APPLIED AND ENVIRONMENTAL MICROBIOLOGY, Oct. 1985, p. 887–893
0099-2240/85/100887-07$02.00/0

Vol. 50, No. 4

Occurrence, Growth, and Suppression of Salmonellae in Composted Sewage Sludge

DAVID HUSSONG,[1]† WYLIE D. BURGE,[2]* AND NANCY K. ENKIRI[2]

Maryland Environmental Service[1] and Soil-Microbial Systems Laboratory, Agricultural Research Service, U.S. Department of Agriculture,[2] Beltsville, Maryland 20705

Received 1 March 1985/Accepted 28 June 1985

Composted sewage sludge may be used to improve soil quality, but there remains some doubt concerning the microbiological safety of the product. Sewage sludge composts from 30 municipalities were sampled, and four samples (12%) contained salmonellae (two contained fewer than 0.3/g, and the other two had 21/g and 1.7 × 10^4/g). All 30 composts were inoculated with salmonellae; the populations decreased at a specific death rate of about 0.15 h^{-1} over 24 h at 36°C. In irradiation-sterilized composts inoculated with salmonellae, the salmonellae grew at a rate of 0.65 doublings per h for over 24 h. Growth and death rates were found to be moisture and flora associated. The growth or death rates for antibiotic-resistant salmonellae were not different from those of nonresistant strains. It was concluded that the active indigenous flora of compost establishes a homeostatic barrier to colonization by salmonellae, and in the absence of competing flora, reinoculated salmonellae may grow to potentially hazardous densities. The active microflora of moist composts eliminated contaminating salmonellae (10^5/g) after 6 weeks.

Municipal sewage sludge contains many microorganisms, including *Salmonella* sp., which may present health risks to the general population (5). To provide a microbiologically safe product, sewage sludge can be composted by the Beltsville method to achieve temperatures over time that kill most pathogens (3). A temperature of 55°C in the portion of the pile exhibiting the minimum pile temperature for a period of 2.5 days will provide adequate destruction of pathogenic microorganisms for release of the compost for use by the general public (3).

Although proper composting kills salmonellae, sterilized composted sludge supports their growth (2, 8), and there have been anecdotal reports of salmonellae in unsterilized marketed composts. Because many vectors (such as infected birds and other animals and heavy equipment used in the composting process) might serve to introduce salmonellae into finished compost, it was necessary to determine whether reinoculation of finished compost with salmonellae would result in a hazardous product. Data obtained from work with raw sludge may have implications for regrowth in compost. Salmonellae do not grow extensively when inoculated into raw sludge containing autocthonous microflora, and mixed coliforms once established in a sterilized sludge will greatly inhibit the growth of inoculated salmonellae (9). Work with compost from a single site indicated that a moisture content of 20% and a carbon-nitrogen ratio in excess of 15:1 was necessary for repopulation (8). However, the effect of moisture may vary with composts, and the carbon-nitrogen ratio will be greatly influenced by the relatively inert carbon of wood chips, a factor that can be expected to vary greatly from compost to compost. Therefore, it was necessary to obtain experience with a number of composts to test the general applicability of the above observations and to establish the relative importance in composts of the factors known to influence the growth of salmonellae in other media. These factors include pH, water activity, temperature, available nutrients, and antagonistic effects owing to the presence of other organisms. We present data showing growth and death of *Salmonella* sp. in composts in the laboratory and the level of salmonellae in various composts throughout the United States.

MATERIALS AND METHODS

Compost sample evaluation. Sewage sludges composted by the Beltsville aerated pile method were shipped to our laboratory in sealed 5-gallon (ca. 19-liter) containers. Thirty facilities provided 31 composts. Coarse material (wood chips) were removed by a mesh screen (0.6 cm). Waterlogged composts were drained overnight at room temperature to permit screening. Moisture content was then determined by drying a preweighed subsample at 95°C overnight and reweighing. Composts were then assayed for *Salmonella* sp., and the remaining portion of the sample was stored (short-term storage) at 4°C in loosely sealed plastic bags. Storage beyond one week was at −20°C.

Sample pH was determined by suspending 1 part sample in 2 parts (wt/vol) distilled water. A dew point microvoltmeter (Wescor, Inc., Logan, Utah) connected to a thermocouple psychrometer (Decagon Devices, Pullman, Wash.) calibrated against saline of known molality was used in an attempt to estimate water activity of six samples of fresh compost. However, after moisture adjustment of composts, water activity data were not linear, and this method was discontinued.

The most probable number (MPN) assay for salmonellae in compost has been previously described (7). Briefly, it involves enrichment in buffered peptone broth, selective enrichment in tetrathionate broth with added brilliant green, selective differentiation on modified xylose-lysine brilliant green agar (mXLBG), presumptive screening on triple sugar-iron agar, and confirmation with slide agglutination assays. Our 5-tube assay detects 0.2 or more salmonellae per g of compost, and the 3-tube assay detects at least 0.3 salmonellae, per g (1). The qualitative assay detects salmonellae in at least 14 g of compost (7). The first 17 composts were tested by the MPN assay by using brilliant green agar and bismuth-

* Corresponding author.
† Present address: Office of Biologics, U.S. Food and Drug Administration, Bethesda, MD 20205.

TABLE 1. Colony counts on various media for distilled water suspensions of *S. newport* 413 showing increased recovery with XL and plate-count agar base

Trial	Medium	Colony counts on:			
		Pour plates		Spread plates	
		CFU/ml (±SD)[b]	Plasmid frequency[c]	CFU/ml (±SD)[b]	Plasmid frequency[c]
A	XL+	$4.7 \times 10^7 \pm 2.6 \times 10^6$	0.79	$5.0 \times 10^7 \pm 2.1 \times 10^6$	0.82
	XL	$5.9 \times 10^7 \pm 2.5 \times 10^6$		$6.1 \times 10^7 \pm$ ND[d]	
	PCA+	$6.0 \times 10^7 \pm 9.8 \times 10^6$	0.83	$4.8 \times 10^7 \pm 1.0 \times 10^7$	0.89
	PCA	$7.2 \times 10^7 \pm 5.7 \times 10^6$		$5.4 \times 10^7 \pm 3.5 \times 10^6$	
B	XL+	$1.0 \times 10^6 \pm 1.1 \times 10^4$	1.00	$6.9 \times 10^5 \pm 3.65 \times 10^4$	0.90
	XL	$8.8 \times 10^5 \pm 7.0 \times 10^4$		$7.7 \times 10^5 \pm 6.4 \times 10^4$	
	NA+	$1.1 \times 10^4 \pm 8.7 \times 10^3$	0.011	$3.3 \times 10^3 \pm 2.3 \times 10^3$	0.005
	NA	$9.6 \times 10^5 \pm 4.4 \times 10^4$		$7.2 \times 10^5 \pm 4.7 \times 10^4$	

[a] PCA, Plate-count agar. NA, Nutrient agar. XL is described in Materials and Methods. +, Media containing antibiotics; see the text.
[b] Standard deviation from three plates.
[c] Plasmid frequency as determined by the proportion of colonies counted on antibiotic-containing media.
[d] ND, No data; two countable plates were averaged.

sulfite agar (4). Subsequent tests used mXLBG agar (7). The first 17 samples were subsequently retested for MPN by using mXLBG, but after freezer storage. Fecal coliform MPN assays were performed as described elsewhere (1).

Sample preparation for regrowth and die-off assay. For compost incubation studies, samples were adjusted to 45% water content by air drying or atomizing sterile distilled water over the compost and aseptically mixing as necessary. Samples (20 g [dry weight]) of compost were placed in 30-dram (ca. 53-g) plastic (snap cap) vials wrapped in foil. Preliminary studies showed that the foil prevents condensation of water vapor on the interior vial walls (presumably by reflecting radiant energy that would cause differential heating of the dark compost mass and result in evaporation of water and condensation on the cooler interior vial wall). Sterilization of composts was achieved by 3 Mrad of irradiation, which presumably has little effect on growth factors other than thorough elimination of competitive microflora. Air exchange was provided through a sterile, cotton-plugged 18- to 20-gauge syringe needle that was pushed through the bottle cap (just after inoculation). Composts were packaged, irradiated, inoculated, and incubated within 1 week. For the preliminary trial and for long-term incubation, air-dried composts (ca. 10% moisture) were also prepared.

Salmonella cultures for regrowth–die-off assay. *Salmonella typhimurium* ATCC 14028 and antibiotic-resistant strain *Salmonella newport* 413 were grown separately for 24 h at 36°C in 150 ml of tryptic soy broth (Difco Laboratories) in a shake-type water bath. *S. newport* was routinely subcultured on media containing 25 μg of ampicillin and 75 μg of tetracycline per ml before broth inoculation (7).

Each fully grown culture was washed by centrifugation at 17,000 × *g* for 10 to 15 min, suspended in chilled 0.5% saline, washed again, and suspended in chilled distilled water. Optical density at 420 nm was used to estimate cell density just before the inoculation of composts. Culture densities were adjusted by diluting with distilled water to achieve ca. 6×10^7 *S. typhimurium* per ml and ca. 2.5×10^7 *S. newport* per ml. Spread-plate inoculation on plate-count agar (Difco), nutrient agar (Difco), or mXLBG agar was used to enumerate salmonellae when composts were inoculated and to confirm densities derived from the optical density at 420 nm. Equal parts of the adjusted suspensions were combined, 10 μl was aseptically planted on the top-center surface of each test compost, and the vial was recapped and incubated. Previous attempts to distribute the inoculum within the sample were too time consuming and prone to sample contamination.

Regrowth and die-off assays. For preliminary studies, irradiated and nonirradiated samples of compost 6175 were inoculated and incubated at 36°C, and one vial taken from each treatment was tested for salmonellae after 7, 15, 24, and 36 h. Subsequent short-term incubations were sampled at 24 h and 7 days for the first 15 samples and only at 7 days thereafter. Long-term incubation study composts were similarly treated but sampled at 1, 3, and 6 months of incubation at room temperature.

For each inoculation series, plate counts were prepared of each strain. Additionally, *S. newport* 413 was plated on XL agar base (Difco) with hydrogen sulfide indicators, ampicillin (25 μg/ml), tetracycline (80 μg/ml), and kanamycin (80 μg/ml) added (XL+) to estimate plasmid (R factor) loss (normally less than 20% and numerically within the standard error of the mean of the plate count). We had observed variability in plate counts of the resistant strain on nutrient agar amended with antibiotics; consistently higher counts were obtained from XL+, which was used thereafter in the preliminary studies (Table 1).

At each sample time, the compost subsample was removed from its vial and diluted 1/10 in chilled buffered peptone. Serial decimal dilutions were prepared, and inocula (0.1 ml) were spread onto mXLBG and XL+ agar plates. Additionally, buffered peptone-enrichment MPN broths were inoculated, and all media were incubated at 36°C for 18 to 24 h. Plate counts showing too few salmonellae (fewer than 25 on plates representing a 10^{-2} final dilution) were discarded, and their MPN analyses were continued to estimate densities of 0.2 to 240/g (in 3-dilution MPN assays). *S. newport* was enumerated on XL+ agar in both plate counts and MPN tests. Because *S. newport* was inoculated as 25% of the total population, the difference between the total salmonellae counted on mXLBG and the resistant salmonellae counted on XL+ easily permitted the monitoring of both populations. Because each strain was of a different serogroup and showed minor colonial morphology distinctions, additional cross-checks were available to confirm these data.

Specific growth rate constants (k_g) were calculated for

TABLE 2. Counts of salmonellae incubated in composts for 7 days at 36°C[a]

Sample no.	Content on receipt			Organisms/g				
				Starting	Nonirradiated compost		Irradiated compost	
	Organisms/g	Water (%)	pH		24 h	7 day	24 h	7 day
6175	—	40	6.5	$6.6 \times 10^3/3.3 \times 10^3$	$1.4 \times 10^3/3 \times 10^2$	NT	$3.1 \times 10^8/8.6 \times 10^7$	NT
6236	—	38	6.8	$5.3 \times 10^4/2.2 \times 10^4$	$3.0 \times 10^3/1.4 \times 10^3$	1.1/0.7	$1.0 \times 10^7/9.4 \times 10^6$	$6.2 \times 10^8/1.9 \times 10^8$
6237	—	39	7.2	$5.3 \times 10^4/2.2 \times 10^4$	$5.7 \times 10^3/5.3 \times 10^3$	1.7/1.0	$8.0 \times 10^6/2.0 \times 10^6$	$3.4 \times 10^7/1.3 \times 10^7$
6238	—	41	5.8	$5.3 \times 10^4/2.2 \times 10^4$	$6 \times 10^2/7 \times 10^2$	—/—	$1.9 \times 10^7/1.1 \times 10^7$	$6.7 \times 10^3/4.1 \times 10^7$
6239	—	37	4.8	$4.3 \times 10^4/2.0 \times 10^4$	1.9×10^3/ND	1.6/1.5	$5.0 \times 10^6/7.9 \times 10^5$	4.5×10^3/ND
6240	—	45	6.8	$5.3 \times 10^4/2.2 \times 10^4$	$6 \times 10^3/5 \times 10^2$	110/3.4	$5.0 \times 10^7/2.6 \times 10^7$	$5.2 \times 10^8/3.3 \times 10^8$
6241	—	48	7.2	$5.3 \times 10^4/2.2 \times 10^4$	$1.3 \times 10^3/1.3 \times 10^3$	0.3/—	$2.2 \times 10^8/4.6 \times 10^7$	$2.1 \times 10^9/1.7 \times 10^8$
6242	—	44	7.2	$5.3 \times 10^4/2.2 \times 10^4$	$1.3 \times 10^3/7 \times 10^2$	1.5/0.4	$1.3 \times 10^7/7.4 \times 10^6$	$3.9 \times 10^8/2.1 \times 10^8$
6243	—	35	6.6	$5.3 \times 10^4/2.2 \times 10^4$	$1.1 \times 10^3/2.0 \times 10^3$	9.3/1.1	$2.0 \times 10^6/9.7 \times 10^5$	$4.1 \times 10^7/2.8 \times 10^7$
6244	—	44	7.6	$5.3 \times 10^4/2.2 \times 10^4$	$1.2 \times 10^3/9 \times 10^2$	9.3/0.4	$6.4 \times 10^6/4.3 \times 10^6$	$1.1 \times 10^8/4.1 \times 10^7$
6246	—	63	4.2	$2.4 \times 10^4/6.5 \times 10^3$	—/—	—/—	ND/ND	—/—
6247	—	22	7.0	$2.4 \times 10^4/6.5 \times 10^3$	$1.9 \times 10^3/3 \times 10^2$	110/4.3	9.5×10^6/ND	$1.0 \times 10^8/1.1 \times 10^4$
6248	—	28	5.9	$2.4 \times 10^4/6.5 \times 10^3$	46/24	—/—	$5.7 \times 10^8/1.6 \times 10^6$	$8.2 \times 10^8/1.1 \times 10^7$
6249	—	54	7.1	$2.4 \times 10^4/6.5 \times 10^3$	3×10^2/110	4.3/1.5	$1.5 \times 10^7/2.0 \times 10^4$	$6.5 \times 10^6/1.0 \times 10^5$
6250	—	40	7.0	$2.4 \times 10^4/6.5 \times 10^3$	ND/2 $\times 10^2$	4.3/—	5.5×10^7/ND	$5.1 \times 10^7/6.8 \times 10^5$
6251	—	47	7.2	$2.4 \times 10^4/6.5 \times 10^3$	$2.1 \times 10^3/3 \times 10^2$	—/—	$3.7 \times 10^6/4.0 \times 10^4$	$4.4 \times 10^7/3.5 \times 10^5$
6252	1.7×10^4	42	NT	$6.0 \times 10^3/5 \times 10^2$	NT	920/—	NT	$7.6 \times 10^7/7.2 \times 10^7$
6253	—	53	6.6	$6.0 \times 10^3/5 \times 10^2$	NT	1.7/0.2	NT	$6.3 \times 10^7/4.3 \times 10^6$
6254	—	48	7.3	$6.0 \times 10^3/5 \times 10^2$	NT	—/—	NT	$2.1 \times 10^7/5.2 \times 10^5$
6255	—	63	5.5	$5.2 \times 10^3/2.0 \times 10^3$	NT	—/—	NT	$1.1 \times 10^7/4.7 \times 10^4$
6256	+(<0.2)	32	7.5	$2.6 \times 10^4/1.6 \times 10^3$	NT	—/—	NT	$2.4 \times 10^8/4.9 \times 10^5$
6257	—	47	7.2	$5.2 \times 10^3/2.0 \times 10^3$	NT	2.3/—	NT	$1.7 \times 10^7/3.1 \times 10^4$
6258	—	28	7.3	$2.6 \times 10^4/1.6 \times 10^3$	NT	—/—	NT	$9.9 \times 10^8/3.9 \times 10^4$
6259	—	42	7.2	$5.2 \times 10^3/2.0 \times 10^3$	NT	—/—	NT	$3.2 \times 10^5/6.7 \times 10^2$
6260	—	37	7.4	$2.6 \times 10^4/1.6 \times 10^3$	NT	—/—	NT	3.2×10^8/ND
6261	—	24	6.9	$6.4 \times 10^3/4.2 \times 10^2$	NT	—/—	NT	$1.6 \times 10^9/2.8 \times 10^5$
6262	21	44	7.6	$2.6 \times 10^4/1.6 \times 10^3$	NT	—/—	NT	$2.2 \times 10^7/1.3 \times 10^5$
6263	—	49	6.8	$2.6 \times 10^4/1.6 \times 10^3$	NT	—/—	NT	$9.4 \times 10^6/3.8 \times 10^5$
6265	+(<0.2)	61	NT	$6.4 \times 10^3/4.2 \times 10^2$	NT	—/—	NT	$1.6 \times 10^7/1.2 \times 10^5$
6266	—	39	5.3	$6.4 \times 10^3/4.2 \times 10^2$	NT	—/—	NT	$2.8 \times 10^8/8.9 \times 10^6$
6267	—	38	7.7	$6.4 \times 10^3/4.2 \times 10^2$	NT	—/—	NT	$8.4 \times 10^8/1.0 \times 10^5$

[a] —, MPN value of less than 0.3 (first 10 samples) or less than 0.2 (remaining samples). +, Qualitative recovery. NT, Not tested. ND, No data (no colonies on plates representing a 10^{-2} final dilution).

[b] Salmonella counts are presented as direct plate counts (greater than 2×10^2) or as MPN counts, representing total salmonellae/antibiotic-resistant *S. newport*.

salmonellae in irradiated composts by first-order kinetics and are therefore expressed as unit time^{-1} (usually hours). Similarly, specific death-rate constants (k_d) were calculated for salmonellae in nonirradiated composts by first-order kinetics and therefore are expressed as unit time^{-1} in hours, days, or weeks as suited to a particular inactivation rate.

RESULTS

Salmonellae were detected in four composted sludges upon receipt (Table 2). Two samples contained salmonellae in qualitative testing only (MPN less than 0.3/g), and two provided MPN values of 21/g (sample 6262) and 1.7×10^4/g (sample 6252). A follow-up inquiry about sample 6252 left doubt concerning the collection method; a second sample (6263) from the same source was free of salmonellae. Fecal coliform densities for samples 6252 and 6263 were 1.3×10^7 and 1.1×10^5/g, respectively. Moisture contents for the composts upon receipt are shown in Table 2. Water activity averaged 0.9953 (±0.0062) for six samples, with average water content of 51% (±5%), and no correlation (0.0454) was observed.

The pH of the samples averaged 6.7 with a standard deviation ($n - 1$ df) of 0.9 (Table 2). Two samples (6239 and 6246) exceeded 2 standard deviation units from the mean.

Of the 17 samples processed by the first MPN method, only sample 6252 yielded salmonellae. After −20°C storage for 1 year and retesting with the mXLBG MPN method (7), again only sample 6252 yielded salmonellae, and it did so at a density of approximately 1 log less (2.4×10^3/g). Coliforms were similarly reduced (6.2×10^6/g) in sample 6252 after freezer storage. The serogroups of the salmonellae recovered from all of the composts were groups B (samples 6252 and 6262), C_1 (samples 6252, 6256, 6256, and 6265), and E (sample 6262).

In the preliminary studies, growth was continuous for 24 h, but the rate of growth was negatively correlated with the tested moisture contents of 45 and 11% (Fig. 1). In nonirradiated composts, salmonella counts declined (Fig. 1).

The seeded salmonellae grew well in all but two of the irradiated composts, reaching about 10^7/g or more at 1 or 7 days. Two of 14 irradiated composts showed declines in salmonellae between 1 and 7 days (Table 2). Of nonirradiated 45% moisture composts, about half did not yield salmonellae after 7 days of 36°C.

Over the 6-month incubation, salmonellae in nonirradiated composts declined several logs in the first month, and in the 45% moisture subset, salmonellae became undetectable thereafter (Fig. 2). Although growth occurred during the first 36 h in irradiated compost 6175 at 11% H_2O, all three air-dried composts showed declines of several logs after 4

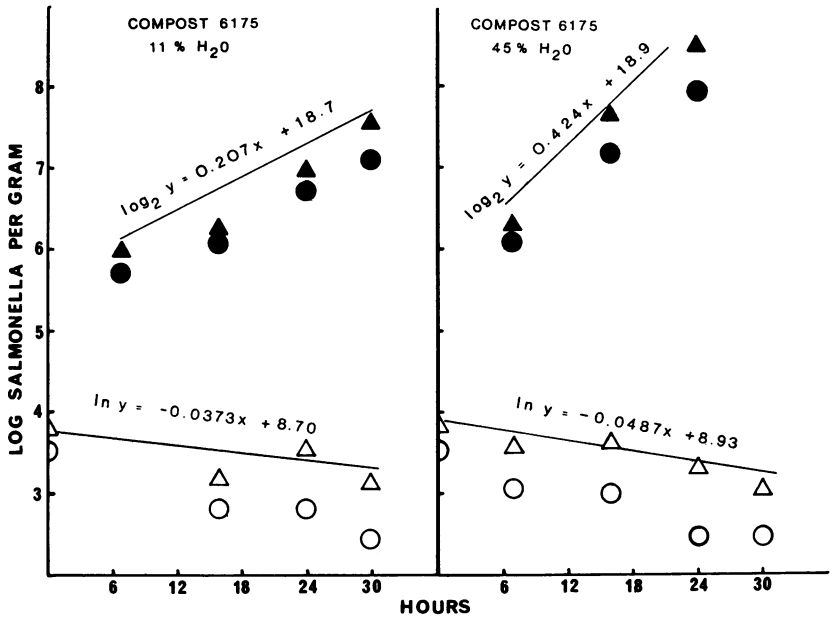

FIG. 1. Growth and death of salmonellae ($\log_{10}$ versus time) over 30 h in compost 6175 at 11 and 45% moisture content. Half symbols on y axes are starting inoculum densities. Triangles are *S. typhimurium*, and circles are *S. newport*. Open symbols represent data from nonsterilized compost, and filled symbols represent data from irradiated (sterile) compost. Growth rate curves shown ($\log_2 Y = \log_{10} Y \times 0.301^{-1}$) were determined for total salmonellae (the sum of both species populations) between 7 and 30 h. Death-rate curves shown were determined for total salmonellae from 0 through 30 h.

weeks, whether irradiated or not (Fig. 3). Antibiotic-resistant salmonellae were no longer detected in dried composts after 4 weeks.

In irradiated, moist (45% moisture) composts, both strains of salmonella achieved ca. 10^8/g (Fig. 1) and then remained at that density for at least 4 weeks (Fig. 2). At 12 weeks, there were fewer antibiotic-resistant than sensitive salmonellae, and with one exception, all salmonella counts had declined. At 26 weeks, about 3 logs of salmonellae was lost, but antibiotic-resistant strains were at approximately equal densities. Antibiotic-resistant salmonellae were always detected as serogroup C_2 (*S. newport*).

Regression analyses of data for air-dried, nonsterile composts and moist, sterile composts up to 26 weeks after inoculation with salmonellae are shown in Tables 3 and 4, respectively. Data for air-dried, irradiated composts over 26 weeks did not permit in-depth analysis. The k_ds (h^{-1} for total salmonella at room temperature for extended incubation

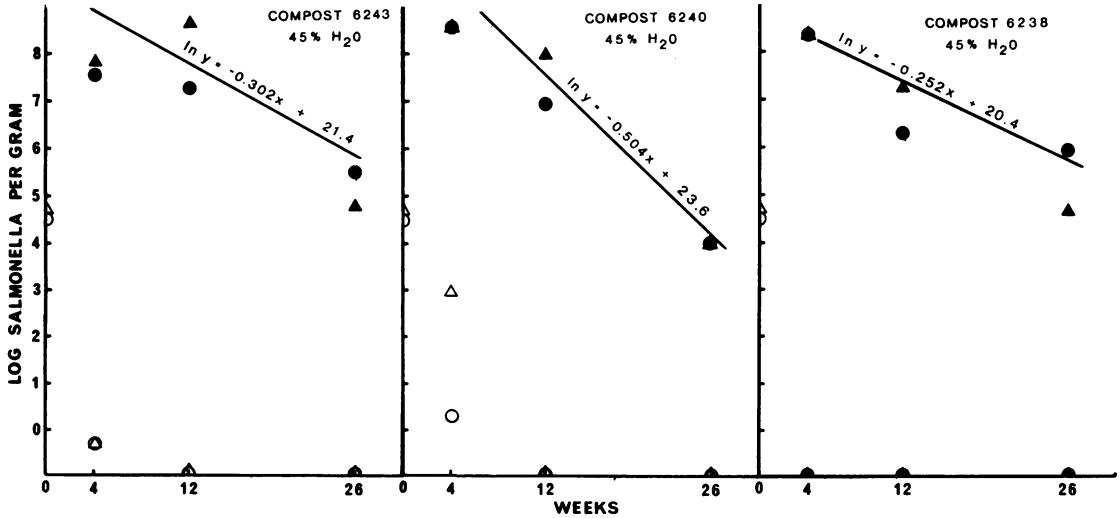

FIG. 2. Growth and death of salmonellae ($\log_{10}$ versus time) over 26 weeks in three composts at 45% moisture content. Symbols are as defined in the legend to Fig. 1. Figures on the x axes indicate "less than" values for undetected salmonellae. Death rates (h^{-1}) shown were determined for total salmonellae (the sum of both species populations) after 4 weeks of growth in irradiated composts. Death rates were too great to be determined for individual nonsterile composts.

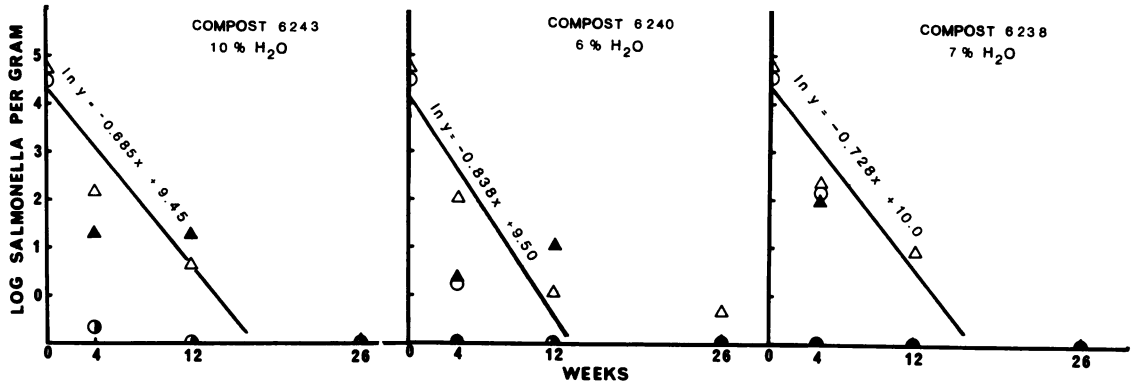

FIG. 3. Growth and death of salmonellae over 26 weeks in three composts at reduced moisture content. Symbols are as defined in the legend to Fig. 1. Figures on the x axes indicate "less than" values for undetected salmonellae. Death rates (h^{-1}) were similar in nonsterile and irradiated composts, and curves for total salmonellae for the moist and dry results of each compost combined are shown.

times in sterile composts were 0.0021 and 0.0049 under moist and dry conditions, respectively; in nonsterile composts, the k_ds were 0.0143 and 0.0029 under moist and dry conditions, respectively (the correlation coefficient was −0.8363, greater than 99% significant at $n = 9$ with 7 df).

DISCUSSION

Of the 31 composts received for study, four (12%) were positive, but only two (6%) provided an MPN value for salmonellae. Examination of compost 6263, from the same source as 6252, made the high salmonella count of 10^4/g appear adventitious. Raw sludge from this sample site was also tested, and salmonellae were undetected. In the presence of 10^7 fecal coliforms per g of sample 6252, competition should have prevented salmonellae (1,000:1 minority) from repopulating. Thus, we suspect these high numbers of salmonellae were due to improper sampling which introduced unusually contaminated material.

Water activity was measured to determine the fraction of water content available for microbial growth. Although this is not an unusual procedure for foods, it does not seem to be readily applicable to composts. Our data did not follow theoretical trends and could not be used.

Preliminary growth testing of irradiated compost 6175 at low moisture showed slightly lower k_gs (0.46 h^{-1}) than at higher moisture (0.65 h^{-1}) over the first 24 h. An initial jump in growth was noted over the first 6 h in both samples (k_g = 1.12 h^{-1} [dry]; k_g = 1.28 h^{-1} [moist]), and this may have been due to the delivery of the inoculum in a 10-μl water suspension, as a spot inoculation. This could have caused a temporary growth spurt from a localized increase in water availability. The slowed growth rates (k_g = 0.207 for dry; k_g = 0.424 for moist) after 7 h tend to support this theory (Fig. 1).

Growth of salmonellae in laboratory-sterilized compost has been previously reported (2). The capacity of 93% of the composts in this study to provide nutrients for growth of salmonellae indicated a great potential for regrowth under these conditions. Conversely, the k_d of salmonellae introduced into nonsterilized composts averaged 0.15 h^{-1} for 12 samples over 24 h. Over 7 days, the k_d slowed to 0.05 h^{-1} for 15 samples, but these data are incomplete because salmonellae were often undetected after 7 days. Thus, salonellae died in nonirradiated compost. The irradiated samples in which salmonellae did not grow were found to be unusually acid, with pH values of less than 5.0.

The 6-month incubation trials used moist (Fig. 2) and dry (Fig. 3) composts and irradiated and nonsterile subsets. All samples were incubated at room temperature (instead of 36°C) to simulate packaged compost under storage and also to save incubator space. Thus, slightly different kinetics were not unexpected. In moist, nonsterile composts, salmonellae persisted through 4 weeks in two of three cases, with loss of viability occurring before 12 weeks. However, in low-moisture composts, salmonellae densities declined in irradiated composts as well as in nonsterile composts. This was in apparent contradiction to data from the preliminary trials in which salmonellae grew to 10^7/g in dry irradiated

TABLE 3. Regression analysis and k_d for total salmonellae in air-dried, nonsterile composts

Compost no.	Incubation time[a]	k_d (h^{-1})	y intercept	ln y intercept	r[c]	Significance (%)[d]	n
6175	0–30 h	0.0373	6.0×10^3	8.70	−0.7747	—	4
6243	0–12 wk	0.00429	2.0×10^4	9.91	NT[e]	NT	3
6240	0–26 wk	0.00246	4.3×10^3	8.34	NT	NT	4
6238	0–12 wk	0.00484	NT	NT	NT	NT	3
6243, 6240, 6238 (combined)		0.00291	8.4×10^3	9.04	−0.8794	99.9	10

[a] Incubation time for points used in linear regression analysis.
[b] k_d, Death rate constant.
[c] r, Correlation coefficient.
[d] Significant value of r, exceeded at n −2 df (80, 90, 95, 99, or 99.9%). —, less than 80%.
[e] NT, Not tested.

TABLE 4. Regression analysis and k_d for total salmonellae in moist sterile composts from 4 through 26 weeks of incubation.

Compost no.	K_d[a] (h^{-1})	y intercept	ln y intercept	r[b]	Significance (%)[c]	n
6243	0.0018	1.9×10^9	21.4	−0.8308	NT[d]	3
6240	0.0030	1.7×10^{10}	23.6	−0.9736	NT	3
6238	0.0015	7.3×10^8	20.4	−0.9986	NT	3
Combined	0.0021	2.9×10^9	21.8	−0.8949	99	9

[a] k_d, Death rate constant.
[b] r, Correlation coefficient.
[c] Significant value of r, exceeded at $n-2$ df (80, 90, 95, 99, or 99.9%).
[d] NT, Not tested.

compost. Had these levels been achieved, higher 4-week densities would have been expected.

By using the death constant for air-dried, nonsterile sample 6175 (Table 3), the starting number of salmonellae (8×10^4/g) should have been undetected in air-dried samples 6243, 6240, and 6238 after 365 h (15 days). However, the k_d observed in these long-term incubations at room temperature was over 10-fold less for both nonsterile ($k_d = 0.0029$ h^{-1}) and sterile ($k_d = 0.0049$ h^{-1}) dry composts, and still the y intercept of the regression analysis was less than the starting inoculum (Table 3). This further reinforced our hypothesis that the salmonellae failed to grow initially at room temperature. However, in moist sterile composts (Fig. 2), salmonellae grew well and then started to die after 1 month. The death rate observed in moist composts containing only salmonellae was the lowest of all but very close to that observed for dry nonsterile composts (see above). Strangely, when dry, sterile composts were inoculated with salmonellae, the observed k_d was higher than for moist sterile or dry nonsterile composts. The death rates for salmonellae in the dry composts (irradiated and nonsterile, combined) showed a change after sample period 1 that may be analogous to that observed in the initial growth of compost 6175 in that the y intercept of the regression analyses shown (Fig. 3) was consistently below the inocula densities. This suggested that the death rates varied over time in dry composts, possibly owing to artifactual changes in moisture content from diffusion of the 10-μl inocula through the samples. Previously (2), samples were inoculated uniformly, but this was too time consuming and not suitable for large numbers of samples.

In moist nonsterile composts, the most rapid loss of salmonellae seemed due to two factors in concert. Because these composts lost salmonellae faster than the others (see above), the single factor of microflora presence was not sufficient to result in rapid loss of salmonellae. Rather, the presence of highly active resident organisms as found in moist, nonirradiated compost seemed most effective in reducing salmonellae after reinoculation. By using the combined k_d for salmonellae in moist, nonirradiated composts ($k_d = 0.0143$ $h^{-1} = 2.40$ $week^{-1}$), it was calculated that 10^5 salmonellae would be undetected (0.1/g or fewer) after 6 weeks of storage. These factors may also account for the earlier observation (9) that salmonellae were more stable in air-dried raw sludge.

An advantage of using *S. newport* 413 as well as *S. typhimurium* arose from the presence of the easy markers (serogroup, antibiotic resistance) to separate them. We tested antibiotic-resistant isolates from laboratory incubations for serogroup and found no serogroup B salmonellae. This suggests that resistance-factor transfer did not occur to a level detectable by our methods, and great hazards owing to the generation of resistant salmonellae (6) may not occur in compost. Although our methods would have detected transfer on a large scale, more sensitive methods exist to test it more precisely. More importantly, had antibiotic resistance been a factor in regrowth suppression, group B salmonellae would have been overrun by resistant strains if the specific antibiotics were present. Because both strains grew rather well in irradiated, sterile composts, growth-suppressing antibiotic or toxic compounds were not present.

Regardless of whether incubation was at 36°C or room temperature, salmonellae grew well in moist, irradiated composts (except in conditions of acid pH) and died in nonirradiated composts. Because the difference between these composts was the presence of other microorganisms, we conclude that competitive influences suppressed salmonellae despite available nutrients. Although low pH composts prevented regrowth, these were atypical composts and may have been less desirable for geochemical reasons. Suggested alternative final treatments for composts have included sterilization by irradiation (2) or other methods. Although this eliminates salmonellae in composts, wherein the potential for reintroduction of the pathogen exists, we believe the resident microflora provide a safety factor. In the absence of these normal flora, reintroduced salmonellae may grow unchecked, thus creating a greater hazard.

Two immediate questions are presented by these findings. They involve the nature of the specific nutrients used by the salmonellae and the identification of the competitive resident microflora that suppress salmonellae. Resolving these additional problems will provide a better understanding of microbial control in the environment.

ACKNOWLEDGMENTS

Although the research (or other work) described in this article has been funded in part by the U.S. Environmental Protection Agency under assistance agreement AD-12-F-2a-029 to the U.S. Department of Agriculture, it has not been subjected to the peer and administrative review of the Agency and, therefore, does not necessarily reflect the views of the Agency, and no official endorsement should be inferred.

Special acknowledgment is made to the composting facility operators and municipalities who graciously provided samples and technical information. The assistance of Wendy Aaronson for characterizing the plasmids in our *Salmonella* spp. cultures is deeply appreciated. We thank Norman Stern of the U.S. Department of Agriculture for graciously providing the antibiotic-resistant salmonellae and Lawrence Bromery of the National Aeronautics and Space Administration for irradiation of samples. Helpful criticisms were accepted from John M. Damare, Edward Dougherty, and Norman Stern.

LITERATURE CITED

1. **American Public Health Association.** 1976. Standard methods for the examination of water and wastewater, 14th ed. American Public Health Association, Washington, D.C.
2. **Brandon, J. R., W. D. Burge, and N. K. Enkiri.** 1977. Inactivation by ionizing radiation of *Salmonella enteritidis* serotype montevideo grown in composted sewage sludge. Appl. Environ. Microbiol. **33**:1011–1012.
3. **Burge, W. D., D. Colacicco, and W. N. Cramer.** 1981. Criteria for achieving pathogen destruction during composting. J. Water Pollut. Control Fed. 53:1683–1690.
4. **Edel, W., and E. H. Kampelmacher.** 1973. Comparative studies on the isolation of "sublethally injured" salmonellae in nine European laboratories. Bull. W.H.O. **48**:167–174.

5. **Geldreich, E. E.** 1972. Water-borne pathogens, p. 207–241. *In* R. Mitchell (ed.), Water pollution microbiology. John Wiley & Sons, Inc., New York.
6. **Holmberg, S. D., J. G. Wells, and M. L. Cohen.** 1984. Animal-to-man transmission of antimicrobial resistant *Salmonella*: investigations of U.S. outbreaks, 1971–1983. Science **225**:833–835.
7. **Hussong, D., N. K. Enkiri, and W. D. Burge.** 1984. Modified agar medium for detecting environmental salmonellae by the most-probable-number method. Appl. Environ. Microbiol. **48:** 1026–1030.
8. **Russ, C. F., and W. A. Yanko.** 1981. Factors affecting salmonellae repopulation in composted sludges. Appl. Environ. Microbiol. **41**:597–602.
9. **Yeager, J. G., and R. L. Ward.** 1981. Effects of moisture content on long-term survival and regrowth of bacteria in wastewater sludge. Appl. Environ. Microbiol. **41**:1117–1122.